A METHOD FOR PREPARING LACTIC ACID FERMENTED SOLUTION OF MUSHROOM AND LACTIC ACID FERMENTED SOLUTION OF MUSHROOM PRODUCED THEREBY

TECHNICAL FIELD

The present invention relates to a method for preparing lactic acid fermented solution of mushroom and a lactic acid fermented solution of mushroom produced thereby, and more particularly, to a method for preparing lactic acid fermented solution of mushroom which comprises the steps of inoculating a lactic acid strain onto a mushroom ingredients-containing medium and fermenting said medium under appropriate conditions and a lactic acid fermented solution of mushroom produced thereby. The lactic acid fermented solution of mushroom thus obtained is excellent in its taste, flavor and gustatoriness, and is effective for inhibiting the formation of peroxidized lipid and the drop of blood sugar level.

BACKGROUND ART

Generally, a mushroom contains lower fat but higher protein and saccharide contents than any other plant. The saccharide also includes trehalose, mannitol, arabinose and the like together with polysaccharide that are hard to absorb into human intestinal tracts and of which main component is an indigestible dietary fiber. Therefore, mushroom is a food material having lower calories than those calculated by food analysis. Furthermore, ergosterol and calcium are commonly included in an amount of 100 · 800mg per an individual, and they are conversed into vitamin D_2 when dried. In addition to said ingredients, mushroom contains vitamin B_1, vitamin B_2 and niacin, not vitamins A and C. Also, as minerals, K is included in a higher portion than Na therein, and P, Ca and Fe follow. Flavorous ingredients of mushroom are mainly nucleic acid and a combination of glutamic acid, succinic acid, malic acid, uric alcohol, etc. Therefore, mushroom is a food material not only having low calories and capable of exerting physiological functions, but also excellent in flavor, taste and gustatoriness.

However, mushroom includes so large amounts of water and nitrogen compounds that it decays and microorganism is easy to propagate due to its soft tissue. Accordingly, the shelf life after harvest of mushroom is short. Mushroom is distributed in the form of live or dried product. Because mushroom is used as a side dish or a flavoring, it plays a role in providing elementary nutrients.

Recently, mushroom has been known to an effective material for inhibiting against cancer and variability, for dropping the formation of lipid in serum, for enhancing immunity to diseases, for inhibiting aging, and for preventing adult diseases. So far, the use of mushroom has been expanded into medicinal field. As such medicinal mushrooms, *Ganderma lucidum*, *Lentinus edodes*, *Pleurotus osteratus*, *Elfvingia applanata*, *agaricus*, *Auricularia auricula* and *Umbilicaria esculenta* have been expected. In particular, it is reported that polysaccharide protein complex included in *Ganderma lucidum* extract exhibits the inhibition of the proliferation of cancer cell, treatment of essential hypertension, inhibition of the formation of peroxidized lipid, etc. Also, *Lentinus edodes* is well known to have anticancer property, dropping effect against cholesterol. tonicity, diuresis, and treatment of hypertension, nephritis, asthma, gastric ulcer, etc. and its extract is reported to have effects for dropping lipid in serum and liver and for inhibiting the liver damage. Also, the polysaccharide extract from *Pleurotus osteratus* were reported to have a dropping effect of cholesterol in serum and an inhibiting effect against liver injuries caused by carbon tetrachloride. Furthermore, an extract obtained from fruit bodies and mycelia of *Pleurotus osteratus* was reported to have an antioxidative effect.

Lactic acid bacterium is a bacterium that produces lactic acid using a carbohydrate such as glucose and lactose, and has been used in fermented milk and cheese from 3,000 B.C. Because of the fact that the milk fermented by lactic acid bacteria inhibits the growth of deleterious bacteria in digestive organs and prevents human aging, lactic acid bacteria fermented milk has been sold as goods worldwide to date. Many beneficial effects of lactic acid bacteria to human health have steadily been studied.

Such effects of lactic acid bacteria include an intestinal regulation (that is, prevention against diarrhea and constipation), suppression against the proliferation of intestinal cancers and aging by inhibiting the growth of deleterious bacteria, promotion of the growth by the formation of vitamins, prevention of adult diseases by controlling cholesterol, reinforcement of immunity, etc.

As typical examples of lactic acid bacteria are included Streptococcus, Pediococcus, Leuconostoc, lactic acid bacillus, vipidus, etc. Lactic acid bacteria are found in the natural world, e.g., digestive tracts of human and animals and almost all of vegetables. Bulgaria bacteria, yogurt bacteria and thermophilus bacteria have been used in the production of yogurt. Yogurt bacteria, casei bacteria and acidophilus bacteria used in the production of beverages containing lactic acid bacteria. Casei bacteria and milk Streptococcus has been used in the production of cheese. Milk Streptococcus has been used in the production of fermented butter. Each specific lactic acid bacterium has been used in the manufacturing processes of different kinds of food.

Lactic acid bacteria inhabit in the intestinal epithelial cell and do their metabolisms. Lactic acid bacteria secrete lactic acid, (lower) fatty acids, bacteriocin, H_2O_2, etc, so as to inhibit the growth of deleterious bacteria and to drop the formation of cholesterol by HMG (Hydroxy Methyl Glutaric), Orotic Acid, Uric Acid, etc, which are formed by the fermentation of lactic acid bacteria. Particularly, *Lactobacillus acidophilous* directly decomposes cholesterol. Lactic acid bacteria activate the microphage detecting bacillus in immune system, thereby detecting the appearance of bacteria and virus, inhibiting the proliferation of cancer cell due to lymphocytic division, increasing the production of Ig A, an antibody in blood, and promoting the production of γ-interferon. Such series of functions improve the immunity, increase nutritional values of food, inhibit the endogenous infection, inhibit the formation of intestinal carcinogens, and derive the death of deleterious bacteria.

Recent changes of diet lead to the high possibilities of diseases in cerebrovascular system, diseases in circulatory system such as heart diseases, hypertension, hyperlipidemia and

arteriosclerosis, and malignant tumors. Such chronic regressive diseases are associated with the disorder of lipid metabolism in a living body. Recently, physiologically active materials to improve human health has vigorously been searched and studied, and as a result, natural ingredients effective against lipid and oxidization have been found and reported. Among them, to edible and medicinal mushrooms have been paid attention as antioxidative materials.

The formation of free radical due to oxidative stress in a living body can peroxidate the biomembrane lipid and the increased peroxidized lipid can do damage to tissues and organs, resulting in metabolism disorder. Therefore, physiologically active matters capable of inhibiting the oxidative damages due to the formation of free radical in a living body are expected to contribute in lowering the occurrence of diseases in circulatory system and chronic diseases such as cancer.

Further, recent improvement of diet and changes of life style lead to fatness due to intake of high-caloric food and shortage of moderate exercise. The advance of industry and complex relationship in society provided the causes of stress. And the development of medicine has prolonged the span of life. These factors are causes of various diseases, particularly diabetes that is a cause.of chronic vascular diseases, and increases the rate of death.

Diabetes mellitus is a disease characterized by inadequate secretion of insulin in pancreas or disorder of insulin receptor in each tissue and by high level of blood sugar. Diabetes mellitus is classified into insulin dependent diabetes mellitus (Type 1 Diabetes mellitus) and non-insulin dependent diabetes mellitus (Type 2 diabetes mellitus). Insulin dependent diabetes mellitus is occurred when pancreas β-cell secreting insulin due to disorder in immune system is destroyed, and non-insulin dependent diabetes mellitus is occurred by disorders in insulin receptor such as muscular cell due to heredity and fatness.

Recent studies for searching various physiologically active materials such as tea tree leaves, coix and mulberry leaves having effects for dropping the blood sugar level have

continuously been progressed. But, these studies have mainly been concentrated on the treatment of type 1 diabetes mellitus.

According to the statistical data, the number of patients with type I diabetes mellitus of the two types is on an increasing trend yearly and, in Korea, 95% of patients with the diabetes mellitus fall on type 2 diabetes mellitus. Agents for dropping blood sugar level are administered orally to patients with type 2 diabetes mellitus together with dietary treatment. To this end, acarbose and voglibose are administered to patients for dropping the blood sugar level after meals, but they are sold at high prices.

To overcome these disadvantages, studies for dropping the blood sugar level using commercially available and edible food or various naturally originating physiologically active materials have been progressed. Korean Pat. No. 165,939 discloses a composition for dropping blood sugar level containing Chinese matrimony extract and a method for preparing the same. Korean Pat. No. 195,886 discloses pharmaceutical composition for treating diabetes mellitus containing *Crdyceps spp* , bezoar. Chinese matrimony, kudzu root, etc, However, the formulations for treating diabetes mellitus disclosed in said patents are not suitable in their taste, flavor and gustatoriness, and their preparing processes are very complicated.

Therefore, needs for a food composition effective for treating type 2 diabetes mellitus and a formulation suitable in taste, flavor and gustatoriness have been existed in this field.

As described above, mushroom and lactic acid bacteria are used as materials for health food, but synergistic effects thereof are not known yet.

DISCLOSURE OF INVENTION

Thus, the present inventors extensively studied to investigate the pharmacological effects of mushroom and lactic acid fermented solution. As a result, we found that lactic acid fermented solution of mushroom exhibits a potent synergistic effect, and then completed the

present invention.

Therefore, it is an object of the present invention to provide a method for preparing lactic acid fermented solution of mushroom excellent in its taste, flavor and gustatoriness and shortened in fermentation period, and a lactic acid fermented solution of mushroom produced thereby.

It is another object of the present invention to provide a method for preparing lactic acid fermented solution of mushroom capable of inhibiting the formation of peroxidized lipid, and a lactic acid fermented solution of mushroom produced thereby.

It is another object of the present invention to provide a method for preparing lactic acid fermented solution of mushroom effective for dropping the blood sugar level, and a lactic acid fermented solution of mushroom produced thereby.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic of the process for preparing lactic acid fermented solution of mushroom according to the present invention.

Fig. 2 is a graph showing the formation rate of the lactic acid fermented solution of *Pleurotus osteratus* obtained in Example 37 according to the present invention.

Fig. 3 is a graph showing the formation rate of lactic acid fermented solution of *Ganderma lucidum* obtained in Example 38 according to the present invention.

Figs. 4a ˙ 4c are graphs showing rheologies of lactic acid fermented solution of *Pleurotus osteratus* obtained in Example 37 according to the present invention.

Figs. 5a ˙ 5c are graphs showing rheologies of lactic acid fermented solution of *Ganderma lucidum* obtained in Example 38 according to the present invention.

Figs. 6a ˙ 6e are graphs showing the change of blood sugar level over the dietary history of lactic acid fermented solutions of *Ganderma lucidum* obtained in Comparative Example 1, Example 40, Example 41, Comparative Example 2 and Example 42, respectively.

Fig. 7 is a graph showing the change of blood sugar level over the dietary history of lactic

acid fermented solution of *Ganderma lucidum* obtained in Example 42 and the dietary history of *Ganderma lucidum* extract.

BEST MODE FOR CARRYING OUT THE INVENTION

A method for preparing lactic acid fermented solution of mushroom according to the present invention comprises the steps of

a) preparing a mushroom ingredients-containing medium;

b) inoculating lactic acid strain onto the medium;

c) culturing the strain-inoculated medium; and

d) aging the cultured medium.

Also, said step (a) comprises the steps of i) obtaining mushroom ingredients from fruit bodies or mycelia of mushroom by grinding or extracting; ii) adding 0.1-10% by weight of the obtained mushroom ingredients, 1-50% by weight. preferably 1-20% by weight of defatted milk, 0.1-20% by weight of sugar and the balance of purified water to the mushroom ingredients-containing medium and homogenizing the mixture to prepare a lactic acid bacteria medium; iii) heat-treating the ingredients-containing medium of mushroom at a temperature ranging 75 ˜ 110°C for 15-40 minutes; and iv) cooling the heat-treated medium to a temperature ranging 35-40°C.

Examples of mushrooms usable in said i) include all of edible and medicinal mushrooms, e.g., *Agaricus blazei*, *Ganderma lucidum*, Grifola frondosa, *Elfvingia applanata*, *Pleurotus osteratus*, *Agaricus bisporus*, Flammulina velutipes, *Lentinus edodes* and *Crdyceps spp.*, but are not limited to them. The parts of the usable mushrooms are fruit bodies and mycelia. In case of *Agaricus blazei*, *Ganderma lucidum*, *Grifola frondosa* and *Elfvingia applanata*, the mushroom ingredients are obtained from their fruit bodies and mycelia are used. And in case of *Pleurotus osteratus*, *Agaricus bisporus*, Flammulina velutipes, *Lentinus edodes* and *Crdyceps spp.*, the mushroom ingredients are obtained their fruit bodies. Also, mushroom ingredient is preferably a mixture of powder and extract from mushroom. In particularly, mushroom ingredient for dropping the blood sugar level is preferably an extract from

Ganderma lucidum.

In step b), 1-10% by weight of lactic acid bacteria in cold storage or heat-treated lactic acid bacteria, based on the total weight of said mushroom ingredients-containing medium, is inoculated onto the medium cooled in iv) of the step a). At this time, the inoculated lactic acid strain is preferably heat-treated lactic acid bacteria in terms with fermentation period. The heat-treatment is carried out by placing the cold-stored strain in an incubator and incubating the strain till a temperature ranging 25-40°C.

In step c), while maintaining the temperature of the incubator within the range of 35-40°C, culturing is carried out for 3-20 hours. In case that the heat-treated lactic acid strain is inoculated, the culture period is preferably for within the range of 3-6 hours.

In step d), aging is carried out at a temperature ranging 3-5°C for a predetermined time.

The present invention also provides a lactic acid fermented solution of mushroom produced by the said method for preparing the lactic acid fermented solution of mushroom.

The property of lactic acid fermented solution of mushroom thus obtained, inhibitory effect against the formation of peroxidized lipid, and dropping effect on blood sugar level are investigated

Agaricus blazei, *Ganderma lucidum*, *Pleurotus osteratus*. *Agaricus bisporus*, Flammulina velutipes, *Grifola frondosa*, *Lentinus edodes*, *Elfvingia applanata* and *Crdyceps spp.* are used to lactic acid fermented solution of mushroom according to the present invention, but the scope of the invention is not limited to them. All of the edible and medicinal mushrooms can be used for preparing the lactic acid fermented solution of mushroom according to the present invention.

First, with reference to Fig. 1, the method for preparing the lactic acid fermented solution of

mushroom according to the present invention is schematically explained.

a) step of preparing a mushroom ingredients-containing medium

i) obtaining mushroom ingredients

One or more parts among the fruit bodies and mycelia of mushroom *Agaricus blazei*, *Ganderma lucidum*, *Grifola frondosa* and *Elfvingia applanata*, and fruit bodies of *Pleurotus osteratus*, *Agaricus bisporus*, *Flammulina velutipes*, *Lentinus edodes* and *Crdyceps spp.* were selected, washed, dried in hot-air drier at a temperature of 60°C and ground to obtain a dried powder of mushroom.

One or more parts among the fruit bodies and mycelia of mushroom *Agaricus blazei*, *Ganderma lucidum*, *Grifola frondosa* and *Elfvingia applanata*, and fruit bodies of *Pleurotus osteratus*, *Agaricus bisporus*, *Flammulina velutipes*, *Lentinus edodes* and *Crdyceps spp.* were selected, washed, and extracted in high-pressured sterilizer using appropriated solvent in a conventional manner to obtain a mushroom extract.

ii) preparing a lactic acid bacteria medium

The medium was prepared by adding 0.1-10% by weight of the obtained mushroom ingredients, 1-50% by weight, preferably 1-20% by weight of defatted milk, 0.1-20% by weight of sugar and the balance of purified water to the mushroom ingredients-containing medium obtained in said i) and homogenizing the mixture to prepare a lactic acid bacteria medium. Other effective components such as oligosaccharide, dextrin, vitamin and mineral can be further included in the lactic acid bacteria medium.

iii ~ iv) heat-treating and cooling

After heat-treating the ingredients-containing medium of mushroom at a temperature ranging 75 ~ 110°C for 15-40 minutes, the heat-treated medium was cooled to temperature ranging 35-40°C.

b) step of inoculating lactic acid strain onto the medium

Lactic acid bacteria in cold storage or heat-treated lactic acid bacteria incubated to a

temperature ranging 25℃ to 40℃ in an incubator was inoculated onto the medium cooled in iv) of the step a). At this time, the amount of the inoculated lactic acid strain can be selected within the range of 1-10% by weight, based on the total weight of said mushroom ingredients-containing medium.

c) step of culturing the strain-inoculated medium

While maintaining the temperature of 35-40℃ in an incubator, lactic acid bacteria medium inoculated with strain was cultured for 3-20 hours. In case that the heat-treated lactic acid strain was inoculated, the culture period was preferably within the range of 3-6 hours.

d) step of aging the cultured medium.

Strain cultured in said step c) was aged at a temperature ranging 3-5℃ for 10 ~ 20 hours.

Hereinafter, the preferred embodiments of the present invention will be described, but the preferred embodiments are intended only for the purpose of an illustrative, and the present invention is not limited thereto.

Example

Material

Mycelia of *Agaricus blazei*, *Ganderma lucidum*, *Grifola frondosa*, *Elfvingia applanata*, *Pleurotus osteratus*, *Agaricus bisporus*, *Flammulina velutipes*, *Lentinus edodes* and *Crdyceps spp.* were cultured in laboratory room, and fruit bodies of *Agaricus blazei*, *Ganderma lucidum*, *Grifola frondosa* and *Elfvingia applanata* were obtained from market.

Examples 1-4: Preparation of lactic acid fermented solution of *Agaricus blazei*

Example 1

Fruit bodies and mycelia of *Agaricus blazei* were ground to obtain a dried powder. A mixture of 5% by weight of the obtained powder, 10% by weight of defatted milk, 2% by weight of sugar and the balance of purified water was added to a medium and homogenized. The mushroom ingredients-containing medium was subject to heat-treatment at a

temperature of 100°C for 20 minutes and then cooled to 37°C.

3% by weight of *Lactobacillus bulgaricus*, based on the total weight of said mushroom ingredients-containing medium, in cold storage was inoculated onto the medium. Six samples of the strain-inoculated mushroom ingredients-containing medium were prepared. While maintaining the temperature of the incubator to 37°C, the samples were cultured for 1, 2, 3, 4, 5 and 6 hours, respectively, and their pH and acidity were measured. Subsequently, the cultured samples were aged at a temperature of 4°C for 12 hours. The aged samples were homogenized with homogenizer to prepare a lactic acid fermented solution of *Agaricus blazei*.

Example 2

Lactic acid fermented solution of *Agaricus blazei* was prepared according to the same procedure as Example 1 except that the *Agaricus blazei* extract was used instead of the dried powder.

Example 3

Fruit bodies and mycelia of *Agaricus blazei* were ground to obtain a dried powder. A mixture of 5% by weight of the obtained powder, 10% by weight of defatted milk, 2% by weight of sugar and the balance of purified water was added to a medium and homogenized. The mushroom ingredients-containing medium was subject to heat-treatment at a temperature of 100°C for 20 minutes and then cooled to 37°C.

Lactobacillus bulgaricus in cold storage was incubated to a temperature of 37°C for 1 hour in an incubator.

3% by weight of the incubated *Lactobacillus bulgaricus*, based on the total weight of said mushroom ingredients-containing medium, was inoculated onto the medium. Six samples of the strain-inoculated mushroom ingredients-containing medium were prepared. While maintaining the temperature of the incubator to 37°C, the samples were cultured for 1, 2, 3, 4, 5 and 6 hours, respectively, and their pH and acidity were measured. Subsequently, the cultured samples were aged at a temperature of 4°C for 12 hours. The aged samples were homogenized with homogenizer to prepare a lactic acid fermented solution of *Agaricus blazei*.

Example 4

Lactic acid fermented solution of *Agaricus blazei* was prepared according to the same procedure as Example 3 except that *Agaricus blazei* extract was used instead of the dried powder.

<u>Examples 5 ~ 8: Preparation of lactic acid fermented solution of *Ganderma lucidum*</u>

Example 5

Lactic acid fermented solution of *Ganderma lucidum* was prepared according to the same procedure as Example 1 except that *Ganderma lucidum* was used instead of *Agaricus blazei*.

Example 6

Lactic acid fermented solution of *Ganderma lucidum* was prepared according to the same procedure as Example 2 except that *Ganderma lucidum* was used instead of *Agaricus blazei*.

Example 7

Lactic acid fermented solution of *Ganderma lucidum* was prepared according to the same procedure as Example 3 except that *Ganderma lucidum* was used instead of *Agaricus blazei*.

Example 8

Lactic acid fermented solution of *Ganderma lucidum* was prepared according to the same procedure as Example 4 except that *Ganderma lucidum* was used instead of *Agaricus blazei*.

<u>Examples 9 ~ 12: Preparation of lactic acid fermented solution of *Pleurotus osteratus*</u>

Example 9

Lactic acid fermented solution of *Pleurotus osteratus* was prepared according to the same procedure as Example 1 except that the fruit bodies of *Pleurotus osteratus* were used instead of the fruit bodies and mycelia of *Agaricus blazei*.

Example 10

Lactic acid fermented solution of *Pleurotus osteratus* was prepared according to the same procedure as Example 2 except that the fruit bodies of *Pleurotus osteratus* were used instead of the fruit bodies and mycelia of *Agaricus blazei*.

Example 11

Lactic acid fermented solution of *Pleurotus osteratus* was prepared according to the same procedure as Example 3 except that the fruit bodies of *Pleurotus osteratus* were used instead

of the fruit bodies and mycelia of *Agaricus blazei.*

Example 12

Lactic acid fermented solution of *Pleurotus osteratus* was prepared according to the same procedure as Example 4 except that the fruit bodies of *Pleurotus osteratus* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Examples 13 ˙ 16: Preparation of lactic acid fermented solution of *Agaricus bisporus*

Example 13

Lactic acid fermented solution of *Agaricus bisporus* was prepared according to the same procedure as Example 1 except that the fruit bodies of *Agaricus bisporus* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 14

Lactic acid fermented solution of *Agaricus bisporus* was prepared according to the same procedure as Example 2 except that the fruit bodies of *Agaricus bisporus* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 15

Lactic acid fermented solution of *Agaricus bisporus* was prepared according to the same procedure as Example 3 except that the fruit bodies of *Agaricus bisporus* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 16

Lactic acid fermented solution of *Agaricus bisporus* was prepared according to the same procedure as Example 4 except that the fruit bodies of *Agaricus bisporus* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Examples 17 ˙ 20: Preparation of lactic acid fermented solution of *Flammulina velutipes*

Example 17

Lactic acid fermented solution of *Flammulina velutipes* was prepared according to the same procedure as Example 1 except that the fruit bodies of *Flammulina velutipes* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 18

Lactic acid fermented solution of *Flammulina velutipes* was prepared according to the same procedure as Example 2except that the fruit bodies of *Flammulina velutipes* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 19

Lactic acid fermented solution of *Flammulina velutipes* was prepared according to the same procedure as Example 3 except that the fruit bodies of *Flammulina velutipes* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

Example 20

Lactic acid fermented solution of *Flammulina velutipes* was prepared according to the same procedure as Example 4 except that the fruit bodies of *Flammulina velutipes* were used instead of the fruit bodies and mycelia of *Agaricus blazei.*

<u>Examples 21 ・ 24: Preparation of lactic acid fermented solution of *Grifola frondosa*</u>

Example 21

Lactic acid fermented solution of *Grifola frondosa* was prepared according to the same procedure as Example 1 except that *Grifola frondosa* was used instead of *Agaricus blazei.*

Example 22

Lactic acid fermented solution of *Grifola frondosa* was prepared according to the same procedure as Example 2 except that *Grifola frondosa* was used instead of *Agaricus blazei.*

Example 23

Lactic acid fermented solution of *Grifola frondosa* was prepared according to the same procedure as Example 3 except that *Grifola frondosa* was used instead of *Agaricus blazei.*

Example 24

Lactic acid fermented solution of *Grifola frondosa* was prepared according to the same procedure as Example 4 except that *Grifola frondosa* was used instead of *Agaricus blazei.*

<u>Examples 25 ・ 28: Preparation of lactic acid fermented solution of *Lentinus edodes*</u>

Example 25

Lactic acid fermented solution of *Lentinus edodes* was prepared according to the same procedure as Example 1 except that the fruit bodies of *Lentinus edodes* were used instead of

the fruit bodies and mycelia of *Agaricus blazei*.

Example 26

Lactic acid fermented solution of *Lentinus edodes* was prepared according to the same procedure as Example 2 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Agaricus blazei*.

Example 27

Lactic acid fermented solution of *Lentinus edodes* was prepared according to the same procedure as Example 3 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Agaricus blazei*.

Example 28

Lactic acid fermented solution of *Lentinus edodes* was prepared according to the same procedure as Example 4 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Agaricus blazei*.

<u>Examples 29 · 32: Preparation of lactic acid fermented solution of *Elfvingia applanata*</u>

Example 29

Lactic acid fermented solution of *Elfvingia applanata* was prepared according to the same procedure as Example 1 except that *Elfvingia applanata* was used instead of *Agaricus blazei*.

Example 30

Lactic acid fermented solution of *Elfvingia applanata* was prepared according to the same procedure as Example 2 except that *Elfvingia applanata* was used instead of *Agaricus blazei*.

Example 31

Lactic acid fermented solution of *Elfvingia applanata* was prepared according to the same procedure as Example 3 except that *Elfvingia applanata* was used instead of *Agaricus blazei*.

Example 32

Lactic acid fermented solution of *Elfvingia applanata* was prepared according to the same procedure as Example 4 except that *Elfvingia applanata* was used instead of *Agaricus*

blazei.

<u>Examples 33 ‐ 36: Preparation of lactic acid fermented solution of *Crdyceps spp.*</u>

Example 33

Lactic acid fermented solution of *Crdyceps spp.* was prepared according to the same procedure as Example 1 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Crdyceps spp.*

Example 34

Lactic acid fermented solution of *Crdyceps spp.* was prepared according to the same procedure as Example 2 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Crdyceps spp.*

Example 35

Lactic acid fermented solution of *Crdyceps spp.* was prepared according to the same procedure as Example 3 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Crdyceps spp.*

Example 36

Lactic acid fermented solution of *Crdyceps spp.* was prepared according to the same procedure as Example 4 except that the fruit bodies of *Lentinus edodes* were used instead of the fruit bodies and mycelia of *Crdyceps spp*

<u>Example 37: Preparation of lactic acid fermented solution of *Pleurotus osteratus*</u>

Lactic acid fermented solution of *Pleurotus osteratus* was prepared according to the same procedure as Example 1 except that a mixture of 1% by weight of the dried powder and 1% by weight of the extract obtained from the fruit bodies and mycelia of *Pleurotus osteratus* was used instead of the dried powder obtained from the fruit bodies and mycelia of *Agaricus blazei*.

<u>Example 38: Preparation of lactic acid fermented solution of *Ganderma lucidum*</u>

Lactic acid fermented solution of *Ganderma lucidum* was prepared according to the same procedure as Example 1 except that a mixture of 0.1% by weight of the dried powder and

5.0% by weight of the extract obtained from the fruit bodies and mycelia of *Ganderma lucidum* was used instead of the dried powder obtained from the fruit bodies and mycelia of *Agaricus blazei*.

Example 39: Preparation of lactic acid fermented solution of mixed mushrooms

A mixture of 1% by weight of the dried powder and 1% by weight of the extract obtained from *Lentinus edodes, Pleurotus osteratus* and *Ganderma lucidum* (weight ratio = 4:4:2), 13% by weight of defatted milk, 10% by weight of oligosaccharide, 1% by weight of dextrin and 74% by weight of purified water was homogenized to produce a mushroom ingredients-containing medium. The produced mushroom ingredients-containing medium was sterilized by heating at a temperature of 80°C for 30 minutes and then cooled to 37°C. Onto the lactic acid bacteria culturing medium thus obtained was inoculated *Lactobacillus bulgaricus* in an amount of 3% by weight based on the total weight of said mushroom ingredients-containing medium. The medium was cultured in an incubator at a temperature of 37°C for 12 hours, and then aged at a temperature of 4°C for 12 hours. The aged medium was homogenized with homogenizer to prepare a lactic acid fermented solution of the mixed mushrooms.

Examples 40 ~ 42, Comparative Examples 1 and 2: Preparation of lactic acid fermented solution of *Ganderma lucidum*

About 1000ml (to 10% of the total weight) of distilled water was added to 100g of *Ganderma lucidum*, and extract using high-pressured sterilizer at a lower temperature than 100°C for 1 hour. The obtained extract was filtered, 100ml of distilled water was added thereto, and extracted at a higher temperature of 121°C for 1 hour again to obtain a *Ganderma lucidum* extract.

0.01% by weight (Comparative Example 1), 0.1% by weight (Example 40), 0.5% by weight (Example 41), 5% by weight (Example 42) and 10% by weight (Comparative Example 2), respectively, of the obtained *Ganderma lucidum* extract, based on the total weight of each medium, were added to each medium consisting of 8% by weight of defatted milk, 10% by weight of oligosaccharide, 1% by weight of dextrin and the balance of purified water

medium, and homogenized.

3% by weight of *Lactobacillus bulgaricus*, based on the total weight of said mushroom ingredients-containing medium, was inoculated onto each medium. While maintaining the temperature of the incubator to 37°C, the each medium was cultured for 12 hours and aged at a temperature of 4°C for 12 hours to obtain lactic acid fermented solutions of *Ganderma lucidum*

Experimental Example

Experimental Example 1: pH, acidity and rheology

To measure pH, acidities and rheologies (hardness, breaking energy, elastic molecule) of lactic acid fermented solutions of mushrooms obtained from Examples 1 ~ 36 over the culture period, samples of lactic acid fermented solutions obtained from each Example were homogenized with a homogenizer at 1000rpm for 30 seconds.

The measurement of pH was carried out with a pH meter, and the measurement of acidity was carried out by taking a portion (10 ml) of each lactic acid fermented solution of mushrooms, diluting with tertiary distilled water at a ratio of 1:1, adding 0.1% phenolphthalein solution thereto and titrating with 0.1N NaOH.

Experimental result:

As shown in Tables 1 and 2, lactic acid fermented solutions of mushrooms obtained from Examples 1-36 had low pH and high acidities. Also, as depicted in Figs. 2-5, effective rate of formation of lactic acid was guarantied and rheologies of hardness, breaking energy and elastic molecule showed soft tissue and excellent properties (e.g., viscosity).

In particular, as shown in Table 2, in case that heat-treated lactic acid bacteria were inoculated onto the mushroom ingredients-containing media, fermentation periods were shortened by about 33%.

Table 1

pH and acidities of lactic acid fermented solutions of mushrooms

	PH/acidity						
	1	3	6	9	12	15	16
Example 1	6.6/0.15	6.2/0.25	5.8/0.36	5.6/0.41	5.1/0.59	4.6/0.74	4.3/0.84
Example 2	6.6/0.15	6.2/0.24	5.7/0.38	5.5/0.44	4.9/0.61	4.6/0.73	4.2/0.87
Example 5	6.6/0.15	6.1/0.28	5.7/0.39	5.5/0.43	4.9/062	4.6/0.74	4.4/0.83
Example 6	6.6/0.15	6.1/0.27	5.8/0.35	5.4/0.47	4.8/0.66	4.5/0.79	4.3/0.85
Example 9	6.6/0.15	6.0/0.30	5.7/0.39	5.4/0.47	4.9/0.62	4.7/0.70	4.5/0.80
Example 10	6.6/0.15	6.2/0.30	5.8/0.36	5.5/0.43	4.9/0.61	4.6/0.72	4.4/0.83
Example 13	6.6/0.15	6.2/0.25	5.7/0.38	5.4/0.46	4.9/0.60	4.6/0.73	4.3/0.86
Example 14	6.6/0.15	6.1/0.27	5.7/0.38	5.4/0.46	4.8/0.66	4.6/0.72	4.4/0.83
Example 17	6.6/0.15	6.2/0.24	5.7/0.39	5.4/0.47	4.9/0.62	4.6/0.74	4.4/0.83
Example 18	6.6/0.15	6.2/0.24	5.8/0.39	5.5/0.44	4.9/0.62	4.7/0.70	4.5/0.80
Example 21	6.6/0.14	6.0/0.29	5.7/0.39	5.3/0.50	4.7/0.70	4.5/0.80	4.2/0.89
Example 22	6.6/0.15	6.2/0.25	5.9/0.33	5.5/0.44	4.8/0.66	4.6/0.74	4.3/0.86
Example 25	6.6/0.15	6.0/0.30	5.7/0.38	5.3/0.49	4.8/0.65	4.6/0.74	4.4/0.82
Example 26	6.6/0.15	6.1/0.28	5.8/0.35	5.4/0.45	4.8/0.65	4.6/0.73	4.4/0.81
Example 29	6.6/0.15	6.3/0.23	5.8/0.35	5.5/0.44	4.9/0.62	4.6/0.74	4.4/0.82
Example 30	6.6/0.15	6.2/0.25	5.9/0.32	5.6/0.40	4.9/0.62	4.6/0.72	4.5/0.79
Example 33	6.6/0.15	6.2/0.24	5.9/0.31	5.5/0.44	4.8/0.66	4.6/0.73	4.4/0.83
Example 34	6.6/0.15	6.2/0.25	5.9/0.33	5 6/0.41	4.8/0.65	4.7/0.70	4.4/0.81

Table 2

pH and acidities of lactic acid fermented solutions of mushrooms

	PH/acidity						
	0	1	2	3	4	5	6
Example 3	6.6/0.15	6.3/0.23	6.1/0.27	5.8/0.36	5.1/0.56	4.8/0.66	4.6/0.74
Example 4	6.6/0.15	6.2/0.25	6.1/0.27	5.7/0.39	5.2/0.52	4.8/0.65	4.6/0.71

Example 7	6.6/0.15	6.4/0.21	6.2/0.25	5.8/0.34	5.0/0.58	4.7/0.70	4.6/0.71
Example 8	6.6/0.15	6.4/0.20	6.2/0.24	5.9/0.33	5.1/0.55	4.7/0.68	4.6/0.72
Example 11	6.6/0.15	6.3/0.23	6.1/0.26	5.8/0.35	5.0/0.57	4.7/0.67	4.7/0.70
Example 12	6.6/0.15	6.4/0.21	6.2/0.24	5.9/0.31	5.2/0.52	4.8/0.65	4.6/0.74
Example 15	6.6/0.15	6.4/0.19	6.2/0.25	6.0/0.30	5.3/0.50	4.9/0.61	4.7/0.70
Example 16	6.6/0.15	6.5/0.17	6.2/0.25	6.0/0.29	5.4/0.47	4.9/0.61	4.7/0.69
Example 19	6.6/0.15	6.5/0.18	6.2/0.25	5.9/0.31	5.3/0.49	5.0/0.59	4.7/0.70
Example 20	6.6/0.15	6.5/0.17	6.2/0.24	5.9/0.31	5.4/0.46	5.0/0.57	4.7/0.68
Example 23	6.6/0.14	6.2/0.24	6.0/0.30	5.8/0.36	5.3/0.50	5.0/0.59	4.7/0.68
Example 24	6.6/0.15	6.1/0.26	5.9/0.32	5.8/0.34	5.3/0.49	5.0/0.57	4.5/0.79
Example 27	6.6/0.15	6.4/0.19	6.1/0.27	5.7/0.39	5.2/0.25	4.8/0.64	4.5/0.76
Example 28	6.6/0.15	6.5/0.17	6.3/0.23	5.9/0.33	5.3/0.50	4.9/0.60	4.6/0.73
Example 31	6.5/0.14	6.4/0.21	6.2/0.25	5.9/0.33	5.6/0.41	5.1/0.56	4.8/0.66
Example 32	6.6/0.15	6.5/0.18	6.2/0.25	6.0/0.30	5.3/0.50	4.9/0.62	4.7/0.69
Example 35	6.5/0.16	6.2/0.25	5.9/0.32	5.7/0.38	5.3/0.49	5.0/0.59	4.6/0.74
Example 36	6.6/0.16	6.3/0.22	5.9/0.33	5.6/0.40	5.2/0.52	4.9/0.60	4.5/0.75

<u>Experimental Example 2: inhibitory effect of peroxidized lipid formation</u>

To investigate the influence of lactic acid fermented solution of mushroom according to the present invention on peroxidized lipid, lactic acid bacteria fermented milk, a mixed powder of *Lentinus edodes*, *Pleurotus ostreatus* and *Ganoderma lucidum*, and lactic acid fermented solution of mushroom obtained in Example 39 were added to cholesterol-containing diet, respectively.

For 4 weeks from the addition, the influences on peroxidized lipid in female rats were investigated in vivo.

① material

The dried products of *Lentinus edodes*, *Ganoderma lucidum* and *Pleurotus ostreatus* were obtained from Jinju Mushroom Agricultural Association. The dried products were cut to

pieces, ground and pulverized to obtain a powder in the form of fine particles passing a screen of 20 mesh.

② experimental animal, condition and diet composition

Female white rats (Sprague Dawley) weighing about 180 g as experimental animals were grown in a condition of temperature 22±2℃, relative humidity 50±5% and under light (07:00 ~ 19:00) and darkness at 12 hour intervals. Feed in the form of solid diet was provided for 1 week and then with normal feed for 4 days. The rats were divided into 5 groups, by 6 rats per one group.

Experimental diet groups consist of normal diet group (ND), cholesterol diet group (CD) (which is obtained by adding 0.5%(w/w) of cholesterol and 0.125%(w/w) of sodium cholate to the normal diet group), lactic acid bacteria fermented milk-supplemented group (CDFM) (which is obtained by adding lactic acid bacteria fermented milk to the cholesterol diet), mushroom powder-supplemented group (CDMP) (which is obtained by adding mushroom powder to the cholesterol diet), and lactic acid fermented solution of mushroom-supplemented group (CDFMMP) (which is obtained by adding lactic acid fermented solution of mushroom to cholesterol diet). At this time, mushroom powder of CDMP group was a mixture of *Lentinus edodes*, *Pleurotus osteratus* and *Ganderma lucidum* and the mixture was added in an amount of 4% to the group. In CDFM group, lactic acid bacteria fermented milk, which is prepared by culturing at a temperature of 30℃ for 12 hours using *Lactobacillus bulgaricus* and by lyophilizing, was added in an amount of 13.5% to the diet. In CDFMMP group, lactic acid bacteria fermented milk, which is prepared by adding a mixture of *Lentinus edodes*, *Pleurotus osteratus* and *Ganderma lucidum* at a ratio of 4:4:2 to a *Lactobacillus bulgaricus* cultured solution in an amount of 4%, by culturing at a temperature of 30℃ for 12 hours and by lyophilizing, was added in an amount of 17.5% to the diet. Experimental diet compositions of these experimental diet groups were shown in Table 3. Experimental diets and drink were freely fed for 4 weeks. Diet feeding amounts were monitored at fixed time every day, and their weights were measured once per a week.

Table 3

Diet composition

	ND	CD	CDFM	CDME	CDFMMP
Casein	20.0	20.0	20.0	20.0	20.0
α-Corn starch	15.0	15.0	15.0	15.0	15.0
Soybean oil	10.0	10.0	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0	5.0	5.0
AIN-93 Mineral Mixture	4.0	4.0	4.0	4.0	4.0
AIN-93 Vitamin Mixture	1.0	1.0	1.0	1.0	1.0
L-Methionine	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2	0.2
Cholesterol	0.0	0.5	0.5	0.5	0.5
Sodium cholate	0	0.125	0.125	0.125	0.125
Fermented milk (FM)	0	0	13.5	0	0
Mushroom extracts (ME)	0	0	0	4.0	0
Lactic acid Fermented Solution of mushroom	0	0	0	0	17.5
Sucrose	balance				

in which

ND: normal diet group

CD: cholesterol diet group

CDFM: lactic acid bacteria fermented milk-supplemented group to the cholesterol diet group

CDME: the mushroom extract-supplemented group to the cholesterol diet group

CDFMME: lactic acid fermented solution of mushroom-supplemented group to the cholesterol diet group

③ sample

At the final experimental day, the experimental animals were fasted for 12 hours, put under slight anesthesia with ether and then taken blood from the inferior vena cava. This blood was centrifuged at room temperature for 15 under 3,000rpm to obtain sera. The incised tissue was washed with cold 0.9% physiological saline water. dried, and weighed to obtain a sample for analyzing the concentration of peroxidized lipid.

④ analyses of serum, liver lipid and blood sugar level

Total cholesterol in serum was measured using Cholesterol C-test wako kit (Wako Junyaku, Osaka, Japan), HDL-cholesterol in serum was measured using HDL-cholesterol E-test wako kit (Wako Junyaku, Osaka, Japan), neutral lipid in serum was measured using Triglyceride E-test wako kit (Wako Junyaku, Osaka, Japan), phospholipid in serum was measured using Phospholipid C-test wako kit (Wako Junyaku, Osaka, Japan), the concentration of glucose in serum was measured using commercially available kit (Wako Junyaku, Osaka, Japan) in accordance with glucose oxidase method, and lipid in liver tissue was extracted in accordance with Folch's method and the concentration thereof was measured the same method as lipid in serum.

⑤ homogenate, microsome and mitochondria fraction of each tissue

1.15% KCl-10 mM phosphate buffer (pH 7.4) was added to each incised tissue, and then homogenized using homogenizer. A part of the solution was taken as a homogenate fraction, and a microsome fraction and mitochondria fraction were separated from the remaining solution, respectively.

⑥ measurement of the concentration of peroxidized lipid in tissue fraction

The concentration of the peroxidized lipid in each tissue was carried out the following method. First, 2ml of thiobarbituric acid (TBA) reagent was added to 1 ml of homogenate, microsome and mitochondria fraction solution, respectively, and mixed well. The mixture was heated in water bath for 15 minutes, cooled, and centrifuged at 3,000 rpm for 15 minutes.

The supernatant was taken and measured absorbance at 535nm. The concentration of peroxidized lipid in tissue was expressed as nmol/g of malondialdehyde.

⑦ Statistics

ALT and AST values in the sera were measured using blood chemistry analyzer (Vitalab, Spectra II, Merck). The statistical significance for the test result of each group was examined based on Student's t-test, and the result was recognized as having a statistical significance when its P value is less than 5%.

The experimental result was expressed as an average value and standard error through one-way ANOVA, the statistical significance for the experimental result of each group was examined based on Duncan's multiple range test, and the result was recognized as having a statistical significance when its P value is less than 5%.

⑧ result

(1) change in concentration of serum lipid

The change in concentration of serum lipid was shown in Table 4.

Table 4

The concentration of lipid and glucose in blood plasma of experimental animal

		ND	CD	CDFM	CDME	CDFMMP
Lipid in serum	Total Cholesterol	60.49±8.11	279.93± 45.62	155.07± 18.60	123.77± 18.55	80.41±9.01
	HDL-Cholesterol	42.25±5.79	16.55±0.88	19.14±1.88	38.55±1.69	41.25±5.99

	Triglyceride	138.17± 4.52	127.28±2.94	113.34±2.69	135.12±2.56	128.47± 2.65
	Phospholipid	101.32± 9.16	131.66±12 14	87.80±11.80	101.76±8.31	97.42±6.52
AI1		0.34±0.03	15.91±1 71	7 10±0 49	2.53±0.06	0.89±0.06
Serum glucose level (mg/100ml)		93.57±8.23	100.11±4.23	81.18±9.69	93.45±4.63	97.63± 11.98

in which

the values mean average±SE for 6 rats per one group.

Atherogenic index (AI) means total cholesterol - HDL cholesterol/ HDL cholesterol, and the values having different characters have statistical significance of $p<0.05$.

As known from Table 4, the concentration of total cholesterol in serum was increased by 4.6 times in cholesterol diet group (CD) when compared with normal diet group (ND). This suggests high cholesterol hyperglycemia. However, the concentration of total cholesterol was decreased by 44.6% in lactic acid bacteria fermented milk-supplemented group (CDFM), 57.4% in mushroom extract-supplemented group (CDMP) and 72.0% in lactic acid fermented solution of mushroom-supplemented group (CDFMMP) when compared with the cholesterol diet group (CD).

The concentration of HDL-cholesterol in serum was increased in CDMP and CDFMMP groups, but has no change in CDFM. Furthermore, the increase was remarkable in CDFMMP group relative to CDMP group. From this result, it seems that lactic acid bacteria have physiologically active ingredients for increasing the concentration of HDL-cholesterol.

According to Framinghan Heart study, when arteriosclerosis index is not more than 3.5, it is safe from the occurrence of coronary artery diseases. Further, the study recommends the index should be maintained to be not more than 4.5.

In Table 3, comparing arteriosclerosis indices between experimental groups, arteriosclerosis

index in cholesterol diet group (CD) was remarkably increased, compared to the normal diet group (ND). And, arteriosclerosis index in cholesterol diet group (CD) was slightly decreased, compared to the lactic acid bacteria fermented milk-supplemented group (CDFM). However, in the mushroom extract-supplemented group (CDMP) and in lactic acid fermented solution of mushroom-supplemented group (CDFMMP), their arteriosclerosis indices were decreased by 68.4% and 83.3%, respectively.

(2) The formation of the peroxidized lipid in biomembrane

Lipid peroxidation in biomembrane is caused by the increased formation of free radical due to oxidative stress in tissue cells and by the decreased antioxidative defense power in a living body. The TBARS levels showing the formation degree of biomembrane peroxidized lipid in animal are enumerated in Table 5.

Table 5

TBARS levels in female rats tissue (nmol/g of tissue)

Ingredients	ND	CD	CDFM	CDMP	CDFMMP
Liver	117.74±4.71^{a}	121.45±3.43^{a}	102.15±9 52^{b}	123 37±10.28^{a}	99.07±5.68^{b}
Heart	25.50±0.37^{a}	24.27±0.42^{ab}	24.16±0 56^{ab}	24.17±0.53^{ab}	23.55±0.67^{b}
Kidney	14.52±0.35^{a}	27.42±0.71b	22.75±2.70^{c}	28.34±0.48^{b}	26.52±1.12^{b}
Spleen	13.74±0.43	13.64±0.42	13.95±0.73	14 60±0.51	14.28±0.65

in which

said values mean average±SE for 6 rats per one group, and

the values having different characters have statistical significance of $p<0.05$.

Table 5 revealed that the relative contents of peroxidized lipid in each tissue were found in liver, kidney, heart and spleen in sequence in the normal diet group and cholesterol diet group. However, in case that male rats were freely fed with cholesterol diet for 4 weeks, the relative contents of TBARS in each tissue were found in brain, kidney, heart, liver and

spleen in sequence. Also in case that male rats (6 months old) were fed with normal diet, the relative contents of TBARS in each tissue were found in heart. liver, brain and kidney in sequence. These results suggested that the formation of peroxidized lipid in tissue depended on the differences between species, age, and diet composition.

Experimental Example 3: the dropping effect of serum glucose level

① method:

To examine the influence of lactic acid fermented solution of mushroom on the drop of serum glucose level, the following method was performed in patients with diabetes mellitus. First, 10 men (age of 25 ˙ 67, average age of 45) having hyperglycemia (average: 315 mg/dL) from normal diet group were selected, and fed with normal diet for 3 weeks. The measurement of serum glucose level was performed at seven before meals. The results were listed in the following Table 6.

Table 6

The change of serum glucose level (mg/dL) in patients with diabetes mellitus as a control group

Days / Patients	1	3	5	7	9	11	13	15	17	19	21
1	342	279	224	275	262	371	197	246	299	303	307
2	295	324	333	287	288	350	401	370	368	362	355
3	378	410	420	387	195	394	338	382	442	351	400
4	254	382	358	299	308	412	382	172	302	296	289
5	324	192	392	373	392	158	381	254	325	285	244
6	313	386	354	411	205	334	309	384	312	306	299

7	312	152	282	201	290	89	342	134	207	281	354
8	321	358	291	198	311	349	299	395	254	322	390
9	310	200	293	254	165	201	257	398	289	271	253
10	300	389	402	352	300	299	380	388	352	327	301

Common yogurt products were fed patients with low serum glucose level in an amount of 200g a day plus normal diet for 3 weeks. The measurement of serum glucose level was performed every morning before meals. The results were listed in the following Table 7. The level was increased to 262 mg/dL (n=9) a week after the feeding, 315 mg/dL (n=7) two weeks after the feeding and 355 mg/dL (n=6) three weeks after the feeding. Accordingly, it was confirmed that there was no dropping effect of serum glucose level in yogurt diet group.

Table 7

The change of serum glucose level (mg/dL) in diabetics fed with yogurt diet

Days / Patients	1	3	5	7	9	11	13	15	17	19	21
1	115	124	109	139	160	228	219	301	340	346	352
2	180	175	198	242	270	292	354	347	395	404	412
3	200	254	380								
4	138	135	124	178	141	185	152	173	195	192	189
5	225	240	290	370	423						
6	192	209	284	319	325	398	425	387			
7	240	290	317	325	354	349	402	372	386	392	398

8	128	119	145	130	135	217	249	299	284	317	350
9	219	260	292	343	392	417	449				
10	208	218	240	315	342	379	315	328	427	429	430

Lactic acid fermented solution of mushroom obtained in Example 42 was fed 10 men with diabetes mellitus who exhibited the same level (321mg/dL) of serum glucose level as control in amount of 200g a day plus normal diet for 3 weeks. The measurement of serum glucose level was performed at seven before meals. The results were listed in the following Table 8. The level was decreased to 207 mg/dL (n=10) a week after the feeding, 166 mg/dL (n=7) two weeks after the feeding and 150 mg/dL (n=10) three weeks after the feeding. Also, the average serum glucose level in 8 of 10 patients with diabetes mellitus was dropped to 121mg/dL (n=8), and in particular, to 123mg/dL 5 days after the feeding.

Accordingly, the results showed that lactic acid fermented solution of mushroom according to the present invention had a significant effect for dropping serum glucose level.

Table 8

The change of serum glucose level (mg/dL) in diabetics fed with lactic acid fermented solution of mushroom

Days patients	1	3	5	7	9	11	13	15	17	19	21
1	342	302	224	295	328	245	201	175	158	142	125
2	295	314	287	254	220	186	195	143	115	113	110
3	405	398	309	253	410	357	231	324	243	447	310
4	292	275	251	269	224	220	228	219	124	141	158
5	215	187	250	149	148	177	136	200	145	134	122
6	400	322	254	159	217	208	178	125	167	158	149
7	338	275	199	228	145	155	101	75	98	99	99

8	216	158	131	69	147	111	92	108	125	114	103
9	295	289	123	118	101	92	97	95	99	100	101
10	411	408	375	275	303	123	298	199	182	244	219

Experimental Example 4: the contents of mushroom extract effective for dropping serum glucose level

To determine the appropriate amounts of mushroom extract, the experiments were carried out using lactic acid fermented solution of mushroom obtained in Examples 40-43 and Comparative Examples 1-2 over 3 persons per each group.

As shown in the following Table 9 and as depicted in Figs. 6a ~ 6e, dropping effect of serum glucose level were insignificant in the concentration ranging from 0.01% to 0.5%, but the serum glucose level was no longer increased in the concentration of not less than 0.1%. Furthermore, the pharmacological effect was most excellent at the level of 5%, but resulted in serious hypoglycemia at the level of not less than 10%. Accordingly, it can be concluded that the optimum amount of mushroom extract as added was about 200g corresponding to the range of 0.1% to 7%. However, it can be varied with sex, age, body weight and severity of diabetes mellitus.

Table 9

The change of serum glucose level (mg/dL) over the contents of lactic acid fermented solution of mushroom in diabetics.

Days Contents/patieuts		1	3	5	7	9	11	13	15	17	19	21
0.01%	1	212	219	230	299	372	402					
	2	107	109	101	98	123	108	127	92	99	104	109
	3	240	279	288	342	319	380	372	399			
	1	244	259	210	149	232	300	198	212	201	211	220

0.1%	2	295	242	302	318	242	115	89	198	242	271	299
	3	192	200	114	75	221	208	182	191	180	167	154
0.5%	1	288	271	249	262	199	190	175	294	205	177	149
	2	314	324	295	270	222	142	157	140	131	126	120
	3	329	245	279	244	309	300	223	258	249	274	299
5%	1	450	430	375	330	250	189	114	79	92	96	99
	2	387	301	254	199	125	100	69	72	89	86	82
	3	394	412	321	249	199	101	82	93	95	92	89
10%	1	324	201	115	99	75	65					
	2	455	437	315	290	142	89	94	115	99	117	135
	3	400	399	387	300	224	202	103	140	109	90	70

The steady intake of lactic acid fermented solution of mushroom according to the present invention helped the serum glucose level be maintained to be normal in most of patients with type 2 diabetes mellitus.

Experimental Example 5: synergistic effect of mushroom extract and lactic acid bacteria

To identify whether the drop of serum glucose level shown in Experimental Examples was occurred by a synergistic effect of mushroom extract and lactic acid bacteria, a patient with diabetes mellitus was continuously fed with mushroom extract and lactic acid fermented solution of mushroom according to the present invention. The intake of lactic acid fermented solution of mushroom according to the present invention and mushroom extract was performed after the measurement of serum glucose level, but before breakfast and supper. The measurement of serum glucose level was performed at seven every two days.

First, lactic acid fermented solution of mushroom obtained in Example 42 was fed a patient for 36 days, and subsequently cut the feeding for 6 days. Thereafter, when the serum glucose level began to increase, the same amount of mushroom extract as lactic acid fermented solution of mushroom was fed the patient. The change of serum glucose level was monitored at intervals of predetermined time (2 days)

As shown in the following Table 10 and accompanying Fig. 7, the serum glucose level was sharply dropped for 10 days, and then stabilized at 100 (mg/dL) for about 20 days (see "A" in Fig. 7). However, since the feeding was cut, the serum glucose level was increased to 276 (mg/dL) (see "B" in Fig. 7).

Subsequently, when the same amount of mushroom extract as lactic acid fermented solution of mushroom was fed the patient, the serum glucose level was slowly decreased. Thereafter, there was no significant change in serum glucose level (see "C" in Fig. 7).

Table 10

The change of serum glucose level (mg/dL) over the intake of lactic acid fermented solution of mushroom and mushroom extract in diabetics.

Days	The change of serum glucose level over lactic acid fermented solution of mushroom	Days	The change of serum glucose level over diet cut	Days	The change of serum glucose over mushroom extract diet
0	302	38	107	44	233
2	247	40	202	46	213
4	203	42	276	48	182
6	161			50	178
8	147			52	170
10	105			54	174
12	95			56	168
14	99			58	179
16	101			60	171
18	104				
20	89				
22	106				

24	107				
26	94				
28	103				
30	99				
32	92				
34	98				
36	96				

From the above results, it can be seen that there was a synergistic effect of mushroom extract and lactic acid bacteria on the drop of serum glucose level.

Lactic acid fermented solution of mushroom produced in accordance with the method according to the present invention is excellent in its taste, flavor and gustatoriness, and effective for inhibiting the formation of peroxidized lipid and the drop of serum glucose level. Accordingly, the present invention is applicable in pharmacology and food industry.

The present disclosure relates to subject matter contained in priority Korean Patent Application Nos. 2001-24513, filed on May 7, 2001, 2001-54236, filed on September 4, 2001, and 2001-73033, filed on November 22, 2001, the contents of all of which are herein expressly incorporated by reference in their entireties.